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Angolan Industry and Chemical Engineering Journal

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Characterization of Borututu (*Cochlospermum angolense welw*) Flower Extracts Obtained by Pressurized Liquid.

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ARTICLE INFO

Keywords:
Borututu,
(*Cochlospermum angolense welw*),
Flowers,
Pressurized Liquid Extraction (PLE),
Antioxidant activity.

medicinal plants are known as natural sources and can explore the various parts (fruit, seed, flower, leaf, stem, root) as nutrients (vitamins, minerals, proteins and fibers) necessary for the correct maintenance of health, which can be used for therapeutic purposes; and/or ingredients in food production, medicines as promising alternatives. The Borututu plant (Cochlospermum angolense welw) is an indigenous tree from Angola, and has great potential as a source of bioactive extracts. Only the root has been used as an infusion by populations in the treatment of malaria due to its hepatoprotective effects. In recent decades, other extraction techniques have been studied with the aim of developing processes to obtain functional extracts from plant matrices. Among the alternatives linked to new technologies, the application of the Pressurized Liquid Extraction Technique (PLE), as an efficient and ecological technique compared to conventional solid-liquid extraction processes, was applied in this study to obtain bioactive extracts from flowers of the borututu plant. Different green/ecological solvents (water, ethanol and ethyl acetate) were evaluated at different extraction temperatures (50-200ºC), in short extraction times (10 minutes). The extracts produced were chemically characterized by colorimetric techniques. The PLE technique allowed high extraction yields to be obtained with less solvent consumption in short periods and with extracts with a high content of phenolic compounds and high antioxidant capacity, surpassing extracts obtained by the traditional method (infusion). This technique allowed ethanol extracts to be obtained at 200°C, generating a high concentration of total phenolic compounds (TPC) and a high yield of 16.27% and 34.26mg/g, respectively. The highest antioxidant capacity was 2029 µmol/g, obtained with water at 150°C. It is concluded that flower extracts demonstrated potential for the production of functional ingredients on an industrial scale.

1. Introduction

Plants have been the main source of medicines in traditional medicine in many cultures around the world, and it is more recently that compounds and possible mechanisms by which these natural sources exert their action have begun to be studied in health. One of the continents that is gaining more and more relevance due to its richness in plant sources and the importance that traditional medicine has had on its population over the centuries is Africa. Borututu (*Cochlospermum angolense welw*) is a plant of the genus Angolense commonly used by rural communities in Angola (Africa) for traditional therapeutic purposes due to its medicinal and edible properties. It is known that flowers and herbal material have been used for centuries and have demonstrated pharmacological properties with anti-inflammatory, antioxidant and antiproferative effects that have been attributed to various polyphenols and the triterpenoid ursolic acid (Szakiel et al., 2013). Furthermore, the presence of isoflavone (kakalide) in dried Pueraria lobata flower extracts was evaluated, which demonstrated beneficial effects as a traditional Chinese herbal remedy for some symptoms of liver damage associated with excessive alcohol intake (Bai et al., 2011).

Among the three endemic African species, C. angolense, C. planchonii and C. tinctorium have attracted the greatest interest due to their hepatoprotective properties and their use in the treatment of malaria (Ferreres et al., 2013). And antiplasmopdial activity has been demonstrated through in vivo and in vitro tests, mainly in the essential oils of root and leaf extracts of C. planchonii and C. tinctorium (Leonardi et al., 2012). Likewise, Presber demonstrated antiplasmodial activity of extracts from the root bark of the species C. angolense in vitro, as well as for inhibition of influenza viruses (Presber et al., 1992) and for the treatment of diseases caused by plasmodium (Presber et al, 1992) and by worms (Presber, 1991ª,199b). On the other hand, the antiviral, anticarcinoma, hepatocellular, hepatoxicity and antioxidant activities of *C. angolense* root bark for the treatment of liver diseases and also for the prophylaxis of malaria associated with the presence of phenolic compounds, total flavonoids, were evaluated in tumor cells. (Pereira. et al., 2014ª).

Liver injuries can be caused by toxic substances, infections and autoimmune diseases, and are among the most serious diseases and one of the main threats to public health (Correia et al., 2015). On the other hand, no less important, malaria is transmitted by a mosquito (anopheles) and is completely preventable and treatable. However, this disease affects 97 countries and territories around the world according to the WHO (Lamien-Meda et al., 2015). Severe complications of plasma falciparum infection cause high morbidity and approximately two million deaths per year (Waknine-Grinbergetal., 2010). Traditional medicines obtained from natural sources continue to be the main source of treatment in most countries where malaria is endemic and many of them have demonstrated antiplasmodial activity with excellent antimalarial results. (Lamien-Meda et al., 2015). It can be noted that only the borututu root (*C. angolense*) is traditionally used for therapeutic practices for the prophylaxis and treatment of malaria and hepatitis by traditional medicine in Angola. Furthermore, it is coincidentally the most studied part of the plant. More in-depth studies of the chemical composition of other parts of the plant, such as flowers and seeds, are suggested, which can facilitate greater discussion about the possible beneficial effects of their consumption on human health, as the reduced knowledge of the composition of plant material makes it difficult to better understanding biological behavior, which would shed more scientific light on its bioactive potential (Stojakowska et al., 2018).

2. Material and Methods

2.1 Materials

Equipment: ASE 350 Dionex Corporation, Sunnyvale, CA, USA, laboratory sieves column - Acero Inox Norma ISO-3310, Knife mill - Retsch Grindomix GM 200, Freeze dryer - Telstar Lyo Beta, Rota vapor - R-210Buchiy Rota vapor Turbo Vap LV Caliper, Spectrophotometer UV-280 SHIMADZU, Spectrophotometer SPECORD210PLUS-analytik jena, Spectrophotometer GENESYS 10SUV-VIS, Spectrophotometer 10UV-TERMO SCIENTIFIC. (TROLOX) 6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylicacid (97%), (DPPH) 2,2-dephenyl-1-picryihdrazyl, fine granulated sand (0.25-30mm), gallic acid (C7H6O5), (Acrylamide BIS) 2%y 30%, sodium thiosulfate (NO2S2O3.5H2O), Sigma-Aldrich. Folin-Ciocalteuss; anhydrous sodium carbonate (Na2Co3) (87%) from gradopharmaceutical, from Panreac, Absolute ethanol (99.5% purity), ethyl acetate (99% purity) from Macron Chromar, MiliQ water from Milipore.

2.1.1. Obtaining Plant Material

The borututu flowers (*Cochlospermun angolense welw*) were collected in the northern region of Angola and dried at room temperature, then packaged and transported to the laboratory.

2.2 Methods

2.2.1. Obtaining Extracts by PLE

Extracts from the flowers were obtained using an accelerated solvent extraction equipment (ASE350 Dionex Corporation, Sunnyvale, CA, USA), equipped with a solvent control unit (Mustafa et al., 2011; Scientific, 2011) according to the diagram as illustrated in Figure 1. Three solvents were used (water, ethanol, ethyl acetate) and four extraction temperatures were studied (50, 100, 150 and 200 °C).

The extraction time was 10 minutes and a fixed pressure of 10 MPa was applied and a single extraction cycle. Extraction cells with a capacity of 10 mL were used and 1 g of plant material was used per extraction. In addition, 1 gram of sea sand was used as a dispersant in each extraction, loaded into the cell along with the plant material in a "sandwich" format as follows: 0.5 g of sea sand plus 1 gram of plant matter plus 0.5 g of sea sand acting as a dispersant. The process of extraction and collection of extracts in collection bottles is carried out automatically. Each extraction process consisted of the following steps: filling the cell with the solvent until reaching the working pressure (10 MPa), heating the solvent (time set automatically by the equipment depending on the extraction temperature), extraction step (10 min). Washing with fresh solvent and purging with nitrogen (these last two steps are carried out automatically to promote the recovery of the extract and its collection in the collection bottle). Extractions were performed in triplicate and frozen. The water was removed by freeze-drying (Telstar Lyo Beta) and the samples were stored at -18ºC in the dark. While ethanol and ethyl acetate were evaporated by Rota steam - R-210 Buchiy Rota steam Turbo.

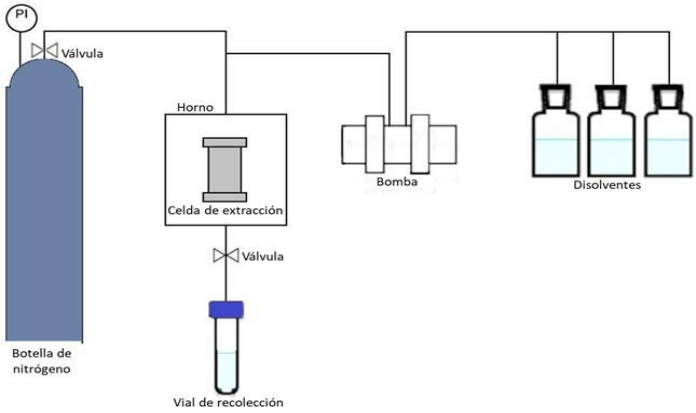


Figure 1. Simplified extraction diagram

Figure 2. ASE 350 DIONEX

2.2.2. Obtaining Extracts by Infusion

The infusion was prepared according to the method published by Pereira et al. (2015). 1 g of plant material was used, immediately added to 200 mL of boiling milliQ water, then stopped and left to rest for 5 min at room temperature. The resulting extract was recovered by vacuum filtration using a kitesate flask. Extractions were performed in triplicate and frozen. The water was removed by freeze-drying (Telstar Lyo Beta) and the samples were stored at -18ºC in a dark environment.

2.2.3. Characterization of Extracts

The first step carried out for the analysis of the obtained strata was to perform a spectral scan at wavelengths between 200-900 nm to determine their absorption spectra. For this, the extracts were diluted with their respective solvents at concentrations of 31.3 µg/ml, 62.5 µg/ml and 125 µg/mL.

The determination of total phenolic compounds (TPC) was carried out using the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999), for which 10L of the extract was diluted in 600L of milli-Q water and then 50 L were added. L of Folin-Ciocalteu reagent and then vortexed and left to rest for one minute. Subsequently, 150L of 20% (w/v) sodium carbonate solution and 190 L of watermili-Q were added. The solutions were kept for 2 hours at room temperature in the dark. After this time, its absorbance was determined at 760 nm. Samples obtained by PLE with water and those obtained by infusion were tested at a concentration of 2 mg/mL. For the rest of the samples, the concentration was 5 mg/mL. Analyzes were performed in triplicate. Gallic acid was used as standard and results were expressed in mg of gallic acid equivalents (GAE) per gram of dry.

The antioxidant activity of the samples was determined using the stable radical DPPH (2,2-dephenyl-1-picryhydrazyl) and the absorbance was recorded at 516 mm using the spectrophotometer (GENESYS 10s UV-VIS). All analyzes were performed in triplicate with the respective blanks as controls. The Trolox curve was obtained by the following equation Y=0.0846x+14.654 and R2=0.9931. The antioxidant capacity of the extracts obtained by PLE and infusion was evaluated through the neutralization of the DPPH radical following the method described by Brand-Williams, Cuvelier and Berset (1995). Dissolving 23.5 mg of DPPH in 100 ml of methanol and the solution was adjusted to have an absorbance of 0.7. For the test, 25L of the extract was added to 975L of the DPPH radical and left to rest at room temperature dark for a while (70 minutes) for the neutralization reaction to develop.

Necessary contact time between the extract and the radical, for the reaction to develop completely. It was determined experimentally for each of the extraction solvents studied. After this time, the absorbance of the samples at 516 nm was determined. For each analysis, a control was used (DPPH radical without extract) and a DPPH radical calibration line was constructed at different concentrations in order to determine the concentration of radical that remained unneutralized for each of the extracts tested.

3. Results and Discussion

3.1 Obtaining Natural Extracts

The natural extracts were obtained using the PLE technique, evaluating the use of three solvents (water, ethanol and ethyl acetate at four temperatures (50 to 200°C) over a period of 10 minutes in static extraction mode and a fixed pressure was applied of 10 MPa, with a washing volume of 50% and a purge time of 90s. For comparison, extracts were obtained by infusion method traditionally used in the preparation of borututu extracts in a solvent/sample ratio of 200ml/g), bringing to boiling adding the dough over the solvent, then stop and leave to rest for 5 minutes. Compared to other varieties of *Cochlospermum*, Borututu has been a little studied plant, and application of the PLE technique has not been explored.

3.2 Extraction yield

The floral extracts obtained a higher extraction yield at 200°C with 47.33% with an increase in temperature and polarity of the solvent as can be seen in Table 1, due to the increase in the solubility of the solutes and also the decrease in the viscosity and surface tension of the solvent with increasing temperature, above the boiling point of the solvent, favoring better penetration of the solvent into the plant matrix (Mustafa et al., 2011; Santos et al., 2012). The extraction yields (extract mass/plant mass) obtained by PLE and infusion are presented in Table 1 and Figure 1, where the dependence on the studied variants is indicated.

Table 1. Extraction yields by PLE and Infusion of flower extracts (g of extract /g plant).

Extract yields (%)			**Temperature (ºC)**			
		Solvente	**50**	**100**	**150**	**200**
PLE	Flowers	Water	17,05 ± 2,4	24,79 ± 6,58	39,44 ± 2,36	47,33 ± 5,69
		Ethanol	6,71 ±0,90	13,21 ± 1,65	17,09 ± 2,30	18,80 ±6,28
		Ethyl acetate	5,62 ± 1,85	8,03 ± 0,60	12,42 ± 0,75	15,44± 1,41
		Infusion flower		18,64 ± 2,3		

And as expected, the yield reduced as the polarity of the solvents decreased, as can be seen in Table 1 and Figure 3.

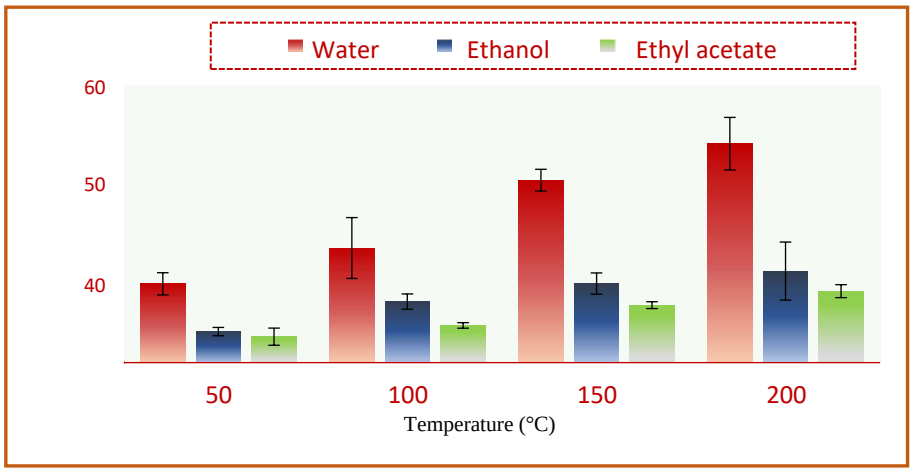


Figure 3. Extraction yield of extracts, polarity and temperature relationship

The PLE technique has been attracting a lot of interest as a promising alternative due to its effectiveness and high extraction yields, which allows for a considerable reduction in solvent consumption and extraction time, as well as obtaining extracts with a high amount of phenolic compounds (Santos et al., 2012; Sumere et al., 2018), demonstrating that the PLE technique was efficient in obtaining extracts with functional activity and high antioxidant capacity (Mustafa et al., 2012; Otero et al., 2019).

3.3 Determination and quantification of total phenolic compounds -TPC

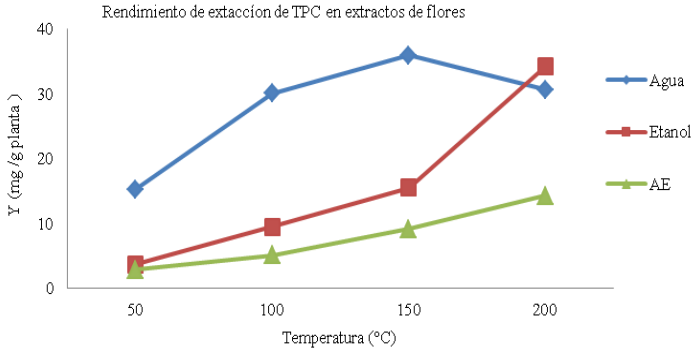
Flower extracts suggest lower concentrations of water-soluble phenolic compounds with higher ethanolic concentrations. These concentrations were obtained at 200ºC in ethanolic extracts, 16.27% being the maximum concentration of TPC. The concentration of TPC using ethanol and ethyl acetate increased with temperature. Likewise, in the case of ethyl acetate, it reached the maximum concentration at 200ºC, which was 11.17%, as seen in Table 2.

Table 2. Concentration of total phenolic compounds (TPC) of flower extracts expressed as % by weight (mg GAE/100 mg extract.

	Solvente	**Temperature (ºC)**			
		50	**100**	**150**	**200**
Flowers	Water	9,27 ± 0,04	11,77 ± 0,07	9,01±0,05	7,06 ± 0, 16
	Ethanol	5,47 ± 0,28	7,25 ± 0,38	9,08 ± 0,16	16,27 ± 0,13
	Ethyl acetate	4,77 ± 0,07	6,96 ± 0,20	7,18 ± 0,06	11,17± 0,16
	Infusión		28,79 ±0,11		

On the other hand, we observed a higher concentration of TPC in extracts obtained by infusion, which indicates a greater presence of water-soluble compounds in extracts under these conditions. The use of temperatures above the boiling point of the solvent, achieved only with the PLE technique, allowed extracts with a high concentration of TPC and TPC extraction yields to be obtained considerably higher than those obtained by infusion. Figure 4 below illustrates the variation in the extraction yield of total phenolic compounds with increasing temperature. Once again, it was possible to observe the TPC extraction yield in ethyl acetate extracts, which were lower (2.92-14.30 mg/g) varying according to temperature (50ºC to 200ºC), while the highest yields were obtained with water in the range between 50ºC and 150ºC (15.23-35.91mg/g) mainly supported by high total extraction yields and not as a consequence of high temperature. These water yields gradually increased with temperature up to 150ºC, from which a slight drop was observed (33.41 mg/g), probably due to the degradation of phenolic compounds as a result of temperature.

Figure 4. Extraction yield of total phenolic compounds (mgTPC/g of plant) in flower extracts



This fact meant that the yield obtained with ethanol at 200ºC (34.16mg/g) was very similar to that obtained with water. In the case of extracts ethanolic, the yield increased linearly between 50 and 150ºC (3.67-15.52 mg/g), followed by of a considerable increase in yield at 200ºC (34.16mg/g).

3.4 Determination of antioxidant activity by the DPPH method

When evaluating the results in Table 3 corresponding to antioxidant activity, organic solvents, in the specific case of ethanol and ethyl acetate, generated TEAC values considerably lower than those obtained by aqueous extracts, all of which were obtained under the same conditions.

Table 3. Antioxidant activity of flower extracts determined by the DPPH method expressed as Trolox equivalents (molTrolox/g extract)

AO/DPPH		Solvente	Temperature (°C)			
			50	100	150	200
PLE	Flores	Wáter	1295±4	1587±2	2029±2	1820±6
		Ethanol	205±21	210±23	671±23	735±9
		Ethyl acetate	99±63	98±23	241±23	171±6
		Infusion		1225±2		

However, the highest TEAC values were achieved with extracts obtained with pressurized water, where the highest TEAC was obtained at a temperature of 150°C (2029mol/g), which was higher than the value achieved by the traditional method, infusion (1225(mol/g). The temperature that allows the extraction of PLE led to obtaining flower extracts with pressurized water, generating higher TEAC values, which meant an increase of 66% as shown in Table 3. When comparing the TEAC values that determine the antioxidant capacity with strata obtained by infusion traditional method (1225(mol/g) with pressurized water at a temperature of 100°C (1587mol/g) this is 23% higher. In this case of water and ethyl acetate, the highest TEAC values were obtained at 150°C (2029 and 241mol/g) respectively and decreased slightly as the temperature increased to 200°C (1820 and 171mol/g) respectively. On the contrary, using ethanol, the highest TEAC value was obtained at the maximum study temperature (735mol/g). Abourashed and Fu (2017) determined the antioxidant capacity using DPPH of a methanolic extract of Borututu root and fractions obtained after fractionation with different organic solvents. Likewise, Pereira et al (2013) observed a positive correlation between the content of phenolic compounds and the antioxidant capacity, determined by DPPH of an extract obtained by infusion of Borututu root with water.

4.Conclusions

In accordance with the proposed objectives, the most relevant conclusions are presented:

a) Extraction yields increased with solvent polarity and extraction temperature, reaching a maximum value at 200ºC of 47.33% in aqueous extracts.
b) The maximum concentration and extraction yield of total phenolic compounds were obtained with ethanol extracts at 200ºC corresponding to 16.27% and 34.16mg/g.
c) The highest antioxidant capacity was obtained at 150ºC with 2029 µmol/g, using water as a solvent. Based on these results, it was demonstrated the high antioxidant capacity of flower extracts obtained by PLE, surpassing those obtained by infusion, with great potential for obtaining ingredients that should be better explored
d) The PLE extraction technique allowed extracts to be obtained with high antioxidant capacity, as well as considerable concentration and yield of phenolic compounds, using water and ethanol as solvents, in just 10 minutes of extraction. These extracts were obtained at a temperature of 150ºC, which is above the boiling point of the solvent; on the contrary, it was not possible to obtain a similar extract.

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